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(FILE 'HOME' ENTERED AT 10:57:30 ON 20 FEB 2007)

FILE 'CAPLUS' ENTERED AT 10:57:42 ON 20 FEB 2007

L1 53660 S PHOSPHOPROTEIN OR PHOSPHOPEPTIDE OR (PHOSPHORYL#(L) (PROTEIN O  
L2 1412 S L1(L) (ISOLAT# OR SEPARAT# OR CHROOMATOGRAPHY)  
L3 89 S L2 AND (GALLIUM OR GA OR IRON OR FE OR ALUMINUM OR AL)  
L4 7 S L3 AND (METAL(L)CHELAT# OR BAPTA OR IDA OR DTPA OR PHENANTHRO

=> d que l4 stat

L1 53660 SEA FILE=CAPLUS ABB=ON PLU=ON PHOSPHOPROTEIN OR PHOSPHOPEPTID  
E OR (PHOSPHORYL#(L) (PROTEIN OR PEPTIDE OR AMINO ACID OR  
CARBOHYDRATE OR LIPID OR PHOSPHATASE OR KINASE))  
L2 1412 SEA FILE=CAPLUS ABB=ON PLU=ON L1(L) (ISOLAT# OR SEPARAT# OR  
CHROOMATOGRAPHY)  
L3 89 SEA FILE=CAPLUS ABB=ON PLU=ON L2 AND (GALLIUM OR GA OR IRON  
OR FE OR ALUMINUM OR AL)  
L4 7 SEA FILE=CAPLUS ABB=ON PLU=ON L3 AND (METAL(L)CHELAT# OR  
BAPTA OR IDA OR DTPA OR PHENANTHROLINE)

=> d 1-7 bib abs

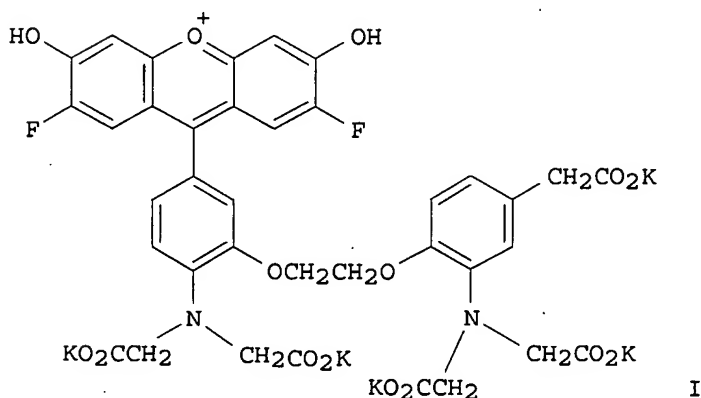
L4 ANSWER 1 OF 7 CAPLUS COPYRIGHT 2007 ACS on STN  
AN 2006:1280218 CAPLUS  
DN 146:137856  
TI Magnetic IDA-modified hydrophilic methacrylate-based polymer  
microspheres for IMAC protein separation  
AU Prikryl, Petr; Horak, Daniel; Ticha, Marie; Kuceroval, Zdenka  
CS Institute of Pathophysiology, 1st Faculty of Medicine and Centre of  
Experimental Hematology, Charles University, Prague, Czech Rep.  
SO Journal of Separation Science (2006), 29(16), 2541-2549  
CODEN: JSSCCJ; ISSN: 1615-9306  
PB Wiley-VCH Verlag GmbH & Co. KGaA  
DT Journal  
LA English  
AB Preparation of a new type of magnetic non-porous poly(2-hydroxyethyl  
methacrylate-co-glycidyl methacrylate) microspheres with hydrophilic  
properties containing coupled iminodiacetic acid (IDA) is described.  
The prepared microspheres were used for the immobilization of Ni(II) or  
Fe(III) ions to show their application in protein binding studies.  
Human IgG was bound to magnetic Ni(II)-IDA-modified microspheres  
and conditions of its adsorption and elution were optimized. Non-specific  
binding of the protein to magnetic microspheres in the absence of Ni(II)  
ions was low. Fe(III) ions immobilized on magnetic IDA  
-modified microspheres were used for the specific binding of porcine  
pepsin, as a model phosphoprotein. The ability of phosphate  
buffer to release the adsorbed enzyme from the microspheres and a low  
adsorption of the dephosphorylated protein indicate the participation of  
phosphate groups in the pepsin interaction. The elaborated method  
represents a rapid technique that can be used not only for the  
separation of proteins but also for anal. purposes.  
RE.CNT 34 THERE ARE 34 CITED REFERENCES AVAILABLE FOR THIS RECORD  
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L4 ANSWER 2 OF 7 CAPLUS COPYRIGHT 2007 ACS on STN  
AN 2005:532184 CAPLUS  
DN 143:149205  
TI Characterisation and evaluation of metal-loaded iminodiacetic acid-silica  
of different porosity for the selective enrichment of phosphopeptides  
AU Trojer, L.; Stecher, G.; Feuerstein, I.; Lubbad, S.; Bonn, G. K.  
CS Institute of Analytical Chemistry and Radiochemistry, Leopold-Franzens  
University, Innsbruck, 6020, Austria  
SO Journal of Chromatography, A (2005), 1079(1-2), 197-207  
CODEN: JCRAEY; ISSN: 0021-9673  
PB Elsevier B.V.  
DT Journal  
LA English  
AB Silica particles of different porosity were functionalized with  
iminodiacetic acid (IDA) and loaded with Fe(III) to  
yield immobilized metal affinity chromatog. stationary phases (Fe  
(III)-IDA-silica) for phosphopeptide enrichment. The  
elution step of bound phosphopeptides was optimized with a <sup>32</sup>P  
radioactive labeled peptide by a comprehensive study. Several elution  
systems, including phosphate buffers of different pH and concentration and EDTA  
solns. were employed. Furthermore the effect of support porosity on  
elution behavior was investigated. Under best conditions recoveries  
higher than 90% were achieved. A solid-phase extraction (SPE) protocol was  
developed for fractionation of phosphorylated and non-phosphorylated  
peptides and desalting of the fractions which is essential for subsequent  
mass spectrometric anal. by the combination of Fe(III)-  
IDA-silica and C18-silica particles. The pH of the loading buffer  
was a critical parameter for the efficiency of the SPE protocol. As tryptic  
digests of  $\alpha$ -lactalbumin, lysozyme and RNase A mixed with three  
synthetic phosphopeptides were fractionated, pH 2.5 provided  
minimal proportion of unspecific bound peptides when comparing the  
fractions after  $\mu$ -LC-electrospray ionization MS separation. The  
effect of a sample derivatization reaction (methylation) on the efficiency  
of phosphopeptide enrichment was further investigated. Blocking  
carboxylate groups by Me ester formation totally prevented unspecific  
interaction with the immobilized Fe(III) ions, but generated  
partially methylated phosphopeptides that increased the  
complexity of the phosphorylated fraction.  
RE.CNT 30 THERE ARE 30 CITED REFERENCES AVAILABLE FOR THIS RECORD  
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L4 ANSWER 3 OF 7 CAPLUS COPYRIGHT 2007 ACS on STN  
AN 2005:146907 CAPLUS  
DN 143:111777  
TI Application of metal-chelate affinity chromatography  
to the study of the phosphoproteome  
AU Imam-Sghiouar, N.; Joubert-Caron, R.; Caron, M.  
CS Laboratoire de Biochimie des Proteines et Proteomique, EA 3408, UFR SMBH  
Leonard de Vinci, Bobigny, Fr.  
SO Amino Acids (2005), 28(1), 105-109  
CODEN: AACIE6; ISSN: 0939-4451  
PB Springer Wien  
DT Journal  
LA English  
AB With the increasing importance of proteome anal., studying the  
phosphoproteome is a priority for functional studies. Therefore, a  
rational approach to simplifying the proteome is needed. In this work, we  
examined the use of immobilized metal affinity chromatog. (IMAC) using  
ferric ions-chelated column for enriching crude cell exts. in  
phosphoproteins. The adsorption of the proteins on Fe<sup>3+</sup> was  
obtained at an acidic pH 5.6, and their elution at a more basic pH in Tris  
buffer. To evaluate the separation, western blots were performed  
with either anti-phosphotyrosine or anti-phosphoserine/threonine. The  
anal. of the eluates demonstrated the selectivity of the separation,  
particularly for proteins phosphorylated on serine or threonine. In  
conclusion, the advantages and the limits of this approach are discussed.  
RE.CNT 13 THERE ARE 13 CITED REFERENCES AVAILABLE FOR THIS RECORD  
ALL CITATIONS AVAILABLE IN THE RE FORMAT

APPLICANT

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
	-----	----	-----	-----	-----
PI	US 2005014197	A1	20050120	US 2004-821522	20040409
	US 2004038306	A1	20040226	US 2003-428192	20030502
	US 7102005	B2	20060905		
	CA 2483868	A1	20040521	CA 2003-2483868	20030502
	AU 2003299466	A1	20040607	AU 2003-299466	20030502
	EP 1546118	A2	20050629	EP 2003-799756	20030502
	R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR, BG, CZ, EE, HU, SK				
	JP 2005539243	T	20051222	JP 2004-549877	20030502
	US 2004171034	A1	20040902	US 2003-703816	20031107
	PRAI	US 2002-377733P	P	20020503	
US 2002-393059P		P	20020628		
US 2002-407255P		P	20020830		
US 2003-440252P		P	20030114		
US 2003-428192		A2	20030502		
US 2003-703816		A2	20031107		
WO 2003-US13765		W	20030502		
OS	MARPAT 142:130341				
GI					

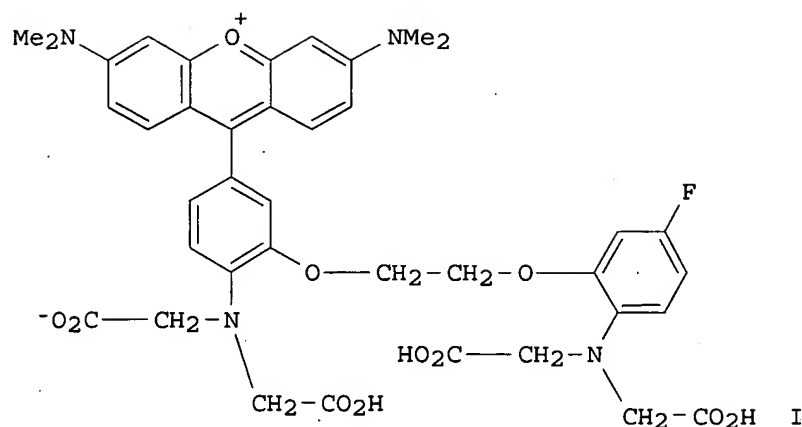


AB The present invention relates to phosphate-binding compds. that find use in binding, detecting and isolating phosphorylated target mols. including the subsequent identification of target mols. that interact with phosphorylated target mols. or mols. capable of being phosphorylated. The phosphate-binding compds. comprise a metal-chelating moiety such as BAPTA, DTPA, IDA, and phenanthroline. This metal-chelating moiety is desirably attached to a label, e.g., a dye or a hapten and/or a reactive group. Preferred dyes are benzofurans, quinazolinones, xanthenes, indoles, benzazoles, and borapolyazaindacenes. A binding solution is provided that comprises a phosphate-binding compound, an

acid and a metal ion wherein the metal ion simultaneously interacts with an exposed phosphate group on a target mol. and the metal chelating moiety of the phosphate-binding compound forming a bridge between the phosphate-binding compound and a phosphorylated target mol. resulting in a ternary complex. The binding solution of the present invention finds use in binding and detecting immobilized and solubilized phosphorylated target mols., isolation of phosphorylated target mols. from a complex mixture and aiding in proteomic anal. wherein kinase and phosphatase substrates and enzymes can be identified. Thus, a compound comprising dihydroxydifluoroxanthene attached to BAPTA and dextran (I) was prepared. I might be used, after addition of  $\text{GaCl}_3$  to form complexes, as an affinity matrix to isolate phosphopeptides. The phosphopeptides might then be identified by mass spectrometry.

L4 ANSWER 5 OF 7 CAPLUS COPYRIGHT 2007 ACS on STN  
 AN 2004:162337 CAPLUS  
 DN 140:213577  
 TI Compositions and methods for detection and isolation of phosphorylated molecules  
 IN Agnew, Brian; Beechem, Joseph; Gee, Kyle; Haugland, Richard; Liu, Jixiang; Martin, Vladimir; Patton, Wayne; Steinberg, Thomas  
 PA Molecular Probes, Inc., USA  
 SO U.S. Pat. Appl. Publ., 83 pp.  
 CODEN: USXXCO  
 DT Patent  
 LA English  
 FAN.CNT 3

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	US 2004038306	A1	20040226	US 2003-428192	20030502
	US 7102005	B2	20060905		
	CA 2483868	A1	20040521	CA 2003-2483868	20030502
	WO 2004042347	A2	20040521	WO 2003-US13765	20030502
	WO 2004042347	A3	20050414		
	W:				
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	RW:				
	GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG				
AU	2003299466	A1	20040607	AU 2003-299466	20030502
EP	1546118	A2	20050629	EP 2003-799756	20030502
	R:				
	AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR, BG, CZ, EE, HU, SK				
CN	1665790	A	20050907	CN 2003-815684	20030502
JP	2005539243	T	20051222	JP 2004-549877	20030502
US	2004171034	A1	20040902	US 2003-703816	20031107
US	2005014197	A1	20050120	US 2004-821522	20040409
PRAI	US 2002-377733P	P	20020503		
	US 2002-393059P	P	20020628		
	US 2002-407255P	P	20020830		
	US 2003-440252P	P	20030114		
	US 2003-428192	A2	20030502		
	WO 2003-US13765	W	20030502		
	US 2003-703816	A2	20031107		
OS	MARPAT 140:213577				
GI					



AB The present invention relates to phosphate-binding compds. that find use in binding, detecting and isolating phosphorylated target mols. including the subsequent identification of target mols. that interact with phosphorylated target mols. or mols. capable of being phosphorylated. A binding solution is provide that comprises a phosphate-binding compound, an acid and a metal ion wherein the metal ion simultaneously interacts with an exposed phosphate group on a target mol. and the metal chelating moiety of the phosphate-binding compound forming a bridge between the phosphate-binding compound and a phosphorylated target mol. resulting in a ternary complex. The binding solution of the present invention finds use in binding and detecting immobilized and solubilized phosphorylated target mols., isolation of phosphorylated target mols. from a complex mixture and aiding in proteomic anal. wherein kinase and phosphatase substrates and enzymes can be identified. A human MRC-5 lung fibroblast cell lysate protein mixture was separated by two-dimensional gel electrophoresis. The gel was fixed and then phosphoproteins were stained with a solution containing 50 mM NaOAc, pH 4.0, 250 mM NaCl, 20% volume/volume 1,2-propanediol, 1  $\mu$ M rhodamine- BAPTA chelating compound I, and 1  $\mu$ M gallium chloride.

RE.CNT 208 THERE ARE 208 CITED REFERENCES AVAILABLE FOR THIS RECORD  
ALL CITATIONS AVAILABLE IN THE RE FORMAT



L4 ANSWER 6 OF 7 CAPLUS COPYRIGHT 2007 ACS on STN

AN 1991:97590 CAPLUS

DN 114:97590

TI Rapid metal-interaction chromatography of proteins and peptides on micropellicular sorbents

AU Bonn, G. K.; Kalghatgi, K.; Horne, W. C.; Horvath, C.

CS Inst. Radiochem., Univ. Innsbruck, Innsbruck, A-6020, Austria

SO Chromatographia (1990), 30(9-10), 484-8

CODEN: CHRGB7; ISSN: 0009-5893

DT Journal

LA English

AB Short columns packed with micropellicular stationary phases consisting of 2- $\mu$ m fused silica microspheres with covalently bound iminodiacetate (IDA) functions at the surface were used for rapid HPLC anal. of proteins by metal-interaction chromatog. (MIC). In contrast to conventional porous stationary phases which elicit relatively long anal. times, the columns packed with sorbents having micropellicular configuration and Ni<sup>2+</sup> or Co<sup>2+</sup> chelated by the IDA functions yielded separation of model proteins in a few minutes with good resolution. A Fe<sup>3+</sup>/IDA column was used for separation of phosphorylated and nonphosphorylated peptides derived from enzymically digested erythrocyte membrane proteins. Stability of the Fe<sup>3+</sup>/IDA column was quite satisfactory as determined by monitoring the iron content of the column effluent and by measuring the amount of iron present in the stationary phase.

L4 ANSWER 7 OF 7 CAPLUS COPYRIGHT 2007 ACS on STN  
AN 1988:507179 CAPLUS  
DN 109:107179  
TI Immobilized metal ion affinity chromatography of proteins on IDA  
-iron(3+)  
AU Sulkowski, Eugene  
CS Dep. Mol. Cell. Biol., Roswell Park Mem. Inst., Buffalo, NY, 14263, USA  
SO Makromolekulare Chemie, Macromolecular Symposia (1988), 17(Int. Symp.  
Affinity Chromatogr. Interfacial Macromol. Interact., 1987), 335-48  
CODEN: MCMSES; ISSN: 0258-0322  
DT Journal  
LA English  
AB Several proteins, selected for their varied isoelec. points within the pH  
range .apprx.4 to .apprx.11, bind to immobilized (chelated to  
iminodiacetate) ferric ion, IDA-Fe<sup>3+</sup>, when applied in a 50 mM  
buffer (pH 6.0). These proteins can be displaced from IDA-Fe<sup>3+</sup>  
columns by an increase of pH from 6 to 8, an increase of NaCl from 0 to  
1M, by both. Apotransferrins, in contrast to other proteins, are able to  
scavenge Fe<sup>3+</sup> from IDA-Fe<sup>3+</sup>. Two proteins, both quite acidic,  
behave quite differently on IDA-Fe<sup>3+</sup>:  $\alpha$ 1-acid glycoprotein  
(sialic acid) does not bind, whereas phosvitin (phosphate!) binds avidly.  
IDA-Fe<sup>3+</sup> sorbent, due to its unusual sorptive properties,  
represents a new addition of particular significance to the family of  
chromatog. sorbents available for protein purification

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L5	22	SEA FILE=CAPLUS ABB=ON	PLU=ON	("AGNEW BRIAN"/AU OR "AGNEW BRIAN J"/AU)
L6	94	SEA FILE=CAPLUS ABB=ON	PLU=ON	("GEE KYLE"/AU OR "GEE KYLE R"/AU)
L7	25	SEA FILE=CAPLUS ABB=ON	PLU=ON	("MARTIN VLADIMIR"/AU OR "MARTIN VLADIMIR V"/AU)
L8	126	SEA FILE=CAPLUS ABB=ON	PLU=ON	L5 OR L6 OR L7

=> d que 19 stat

L5	22	SEA FILE=CAPLUS ABB=ON	PLU=ON	("AGNEW BRIAN"/AU OR "AGNEW BRIAN J"/AU)
L6	94	SEA FILE=CAPLUS ABB=ON	PLU=ON	("GEE KYLE"/AU OR "GEE KYLE R"/AU)
L7	25	SEA FILE=CAPLUS ABB=ON	PLU=ON	("MARTIN VLADIMIR"/AU OR "MARTIN VLADIMIR V"/AU)
L8	126	SEA FILE=CAPLUS ABB=ON	PLU=ON	L5 OR L6 OR L7
L9	1	SEA FILE=CAPLUS ABB=ON	PLU=ON	L8 AND PHOSPHO#

=> d bib abs

L9 ANSWER 1 OF 1 CAPLUS COPYRIGHT 2007 ACS on STN  
 AN 2005:472425 CAPLUS  
 DN 143:22621  
 TI Competitive immunoassay using a ligand analog covalently bonded to a  
 fluorescent reporter molecule  
 IN Beechem, Joseph; Gee, Kyle; Hagen, David; Johnson, Iain; Kang,  
 Hee Chol; Pastula, Christina  
 PA Molecular Probes, Inc., USA  
 SO PCT Int. Appl., 123 pp.  
 CODEN: PIXXD2  
 DT Patent  
 LA English  
 FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 2005050206	A2	20050602	WO 2004-US30711	20040917
	WO 2005050206	A3	20060302		
	W:	AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW			
	RW:	BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG			
	US 2006160068	A1	20060720	US 2004-943463	20040917
PRAI	US 2003-504322P	P	20030917		
	US 2003-505455P	P	20030923		

OS MARPAT 143:22621

AB The present invention provides ligand-detection reagents, ligand analogs and methods for determining the presence of a ligand in a sample. The ligand-detection reagent comprises a ligand-binding antibody and a ligand analog to form an antibody-ligand analog complex wherein the ligand analog is covalently bonded to a reporter mol. This complex may addnl. comprise a labeling protein non-covalently bonded to the antibody to form a ternary complex wherein the labeling protein comprises a monovalent antibody fragment or a non-antibody protein that is covalently bonded to a label moiety. The reporter mol. is either quenched by the ligand-binding antibody or by the label moiety of the labeling protein, depending on the reporter mol. and the ligand-binding antibody, wherein the amount of quenching is directly related to the amount of ligand present in the sample. Alternatively, the ligand analog is fluorogenic wherein the ligand analog is essentially non-fluorescent in solution but when bound by the ligand-binding antibody the detectable signal increases. In this instance a decrease in signal, as opposed to the relieving of quenching, is measured for the presence of a target ligand.

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55075 PHOSPHORYLATED  
L10 9 L8 AND PHOSPHORYLATED

=> d 1-9 bib abs

L10 ANSWER 1 OF 9 CAPLUS COPYRIGHT 2007 ACS on STN  
 AN 2005:472425 CAPLUS  
 DN 143:22621  
 TI Competitive immunoassay using a ligand analog covalently bonded to a  
 fluorescent reporter molecule  
 IN Beechem, Joseph; Gee, Kyle; Hagen, David; Johnson, Iain; Kang,  
 Hee Chol; Pastula, Christina  
 PA Molecular Probes, Inc., USA  
 SO PCT Int. Appl., 123 pp.  
 CODEN: PIXXD2  
 DT Patent  
 LA English  
 FAN.CNT 1

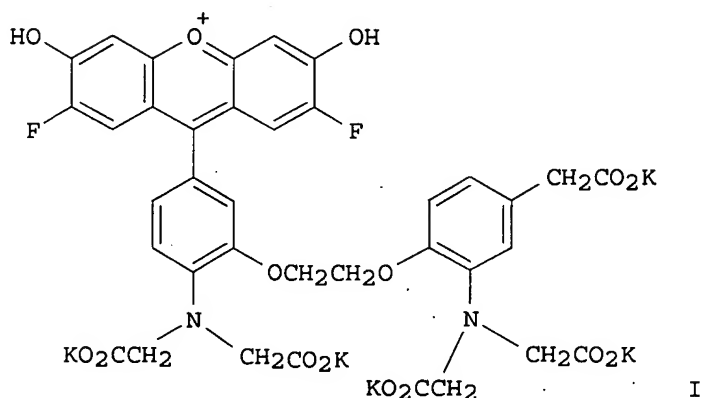
	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 2005050206	A2	20050602	WO 2004-US30711	20040917
	WO 2005050206	A3	20060302		
	W:	AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW			
	RW:	BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG			
	US 2006160068	A1	20060720	US 2004-943463	20040917
PRAI	US 2003-504322P	P	20030917		
	US 2003-505455P	P	20030923		

OS MARPAT 143:22621

AB The present invention provides ligand-detection reagents, ligand analogs and methods for determining the presence of a ligand in a sample. The ligand-detection reagent comprises a ligand-binding antibody and a ligand analog to form an antibody-ligand analog complex wherein the ligand analog is covalently bonded to a reporter mol. This complex may addnl. comprise a labeling protein non-covalently bonded to the antibody to form a ternary complex wherein the labeling protein comprises a monovalent antibody fragment or a non-antibody protein that is covalently bonded to a label moiety. The reporter mol. is either quenched by the ligand-binding antibody or by the label moiety of the labeling protein, depending on the reporter mol. and the ligand-binding antibody, wherein the amount of quenching is directly related to the amount of ligand present in the sample. Alternatively, the ligand analog is fluorogenic wherein the ligand analog is essentially non-fluorescent in solution but when bound by the ligand-binding antibody the detectable signal increases. In this instance a decrease in signal, as opposed to the relieving of quenching, is measured for the presence of a target ligand.

L10 ANSWER 2 OF 9 CAPLUS COPYRIGHT 2007 ACS on STN  
 AN 2005:58103 CAPLUS  
 DN 142:130341  
 TI Metal-binding molecules and metal complexes and methods for detection and isolation of phosphorylated molecules  
 IN Agnew, Brian; Gee, Kyle R.; Martin, Vladimir V.  
 PA USA  
 SO U.S. Pat. Appl. Publ., 96 pp., Cont.-in-part of U.S. Ser. No. 703,816.  
 CODEN: USXXCO  
 DT Patent  
 LA English  
 FAN.CNT 3

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	US 2005014197	A1	20050120	US 2004-821522	20040409
	US 2004038306	A1	20040226	US 2003-428192	20030502
	US 7102005	B2	20060905		
	CA 2483868	A1	20040521	CA 2003-2483868	20030502
	AU 2003299466	A1	20040607	AU 2003-299466	20030502
	EP 1546118	A2	20050629	EP 2003-799756	20030502
	R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR, BG, CZ, EE, HU, SK				
	JP 2005539243	T	20051222	JP 2004-549877	20030502
	US 2004171034	A1	20040902	US 2003-703816	20031107
PRAI	US 2002-377733P	P	20020503		
	US 2002-393059P	P	20020628		
	US 2002-407255P	P	20020830		
	US 2003-440252P	P	20030114		
	US 2003-428192	A2	20030502		
	US 2003-703816	A2	20031107		
	WO 2003-US13765	W	20030502		
OS	MARPAT 142:130341				
GI					



AB The present invention relates to phosphate-binding compds. that find use in binding, detecting and isolating phosphorylated target mols. including the subsequent identification of target mols. that interact with phosphorylated target mols. or mols. capable of being phosphorylated. The phosphate-binding compds. comprise a metal-chelating moiety such as BAPTA, DTPA, IDA, and phenanthroline. This metal-chelating moiety is desirably attached to a label, e.g., a dye or a

hapten and/or a reactive group. Preferred dyes are benzofurans, quinazolinones, xanthenes, indoles, benzazoles, and borapolyazaindacenes. A binding solution is provided that comprises a phosphate-binding compound, an acid and a metal ion wherein the metal ion simultaneously interacts with an exposed phosphate group on a target mol. and the metal chelating moiety of the phosphate-binding compound forming a bridge between the phosphate-binding compound and a phosphorylated target mol. resulting in a ternary complex. The binding solution of the present invention finds use in binding and detecting immobilized and solubilized phosphorylated target mols., isolation of phosphorylated target mols. from a complex mixture and aiding in proteomic anal. wherein kinase and phosphatase substrates and enzymes can be identified. Thus, a compound comprising dihydroxydifluoroxanthene attached to BAPTA and dextran (I) was prepared. I might be used, after addition of  $GaCl_3$  to form complexes, as an affinity matrix to isolate phosphopeptides. The phosphopeptides might then be identified by mass spectrometry.



L10 ANSWER 3 OF 9 CAPLUS COPYRIGHT 2007 ACS on STN

AN 2004:722822 CAPLUS

DN 141:239312

TI Compositions and methods for detection and isolation of phosphorylated molecules

IN Agnew, Brian; Beechem, Joseph; Gee, Kyle; Haugland, Richard; Steinberg, Thomas; Patton, Wayne

PA USA

SQ U.S. Pat. Appl. Publ., 89 pp., Cont.-in-part of U.S. Ser. No. 428,192.  
CODEN: USXXCO

DT Patent

LA English

FAN.CNT 3

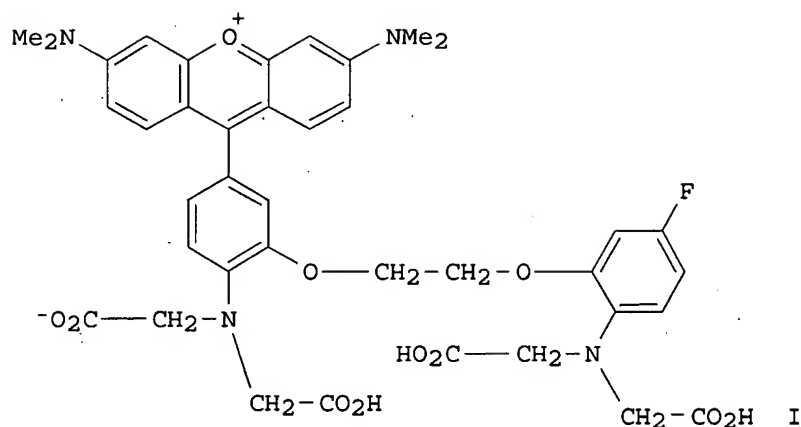
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	US 7102005	B2	20060905		
	CA 2483868	A1	20040521	CA 2003-2483868	20030502
	AU 2003299466	A1	20040607	AU 2003-299466	20030502
	EP 1546118	A2	20050629	EP 2003-799756	20030502
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	US 2005014197	A1	20050120	US 2004-821522	20040409
	WO 2005047901	A2	20050526	WO 2004-US36968	20041105
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PRAI	US 2002-377733P	P	20020503
	US 2002-393059P	P	20020628
	US 2002-407255P	P	20020830
	US 2003-440252P	P	20030114
	US 2003-428192	A2	20030502
	WO 2003-US13765	W	20030502
	US 2003-703816	A2	20031107

AB The present invention relates to phosphate-binding compds. that find use in binding, detecting and isolating phosphorylated target mols. including the subsequent identification of target mols. that interact with phosphorylated target mols. or mols. capable of being phosphorylated. A binding solution is provide that comprises a phosphate-binding compound, an acid and a metal ion wherein the metal ion simultaneously interacts with an exposed phosphate group on a target mol. and the metal chelating moiety of the phosphate-binding compound forming a bridge between the phosphate-binding compound and a phosphorylated target mol. resulting in a ternary complex. The binding solution of the present invention finds use in binding and detecting immobilized and solubilized phosphorylated target mols., isolation of phosphorylated target mols. from a complex mixture and aiding in proteomic anal. wherein kinase and phosphatase substrates and enzymes can be identified.

L10 ANSWER 4 OF 9 CAPLUS COPYRIGHT 2007 ACS on STN  
 AN 2004:162337 CAPLUS  
 DN 140:213577  
 TI Compositions and methods for detection and isolation of  
 phosphorylated molecules  
 IN Agnew, Brian; Beechem, Joseph; Gee, Kyle; Haugland,  
 Richard; Liu, Jixiang; Martin, Vladimir; Patton, Wayne;  
 Steinberg, Thomas  
 PA Molecular Probes, Inc., USA  
 SO U.S. Pat. Appl. Publ., 83 pp.  
 CODEN: USXXCO  
 DT Patent  
 LA English  
 FAN.CNT 3

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	US 2004038306	A1	20040226	US 2003-428192	20030502
	US 7102005	B2	20060905		
	CA 2483868	A1	20040521	CA 2003-2483868	20030502
	WO 2004042347	A2	20040521	WO 2003-US13765	20030502
	WO 2004042347	A3	20050414		
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	GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR,				
	LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH,				
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	GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY,				
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	BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG				
	AU 2003299466	A1	20040607	AU 2003-299466	20030502
	EP 1546118	A2	20050629	EP 2003-799756	20030502
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	AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT,				
	IE, SI, LT, LV, FI, RO, MK, CY, AL, TR, BG, CZ, EE, HU, SK				
	CN 1665790	A	20050907	CN 2003-815684	20030502
	JP 2005539243	T	20051222	JP 2004-549877	20030502
	US 2004171034	A1	20040902	US 2003-703816	20031107
	US 2005014197	A1	20050120	US 2004-821522	20040409
PRAI	US 2002-377733P	P	20020503		
	US 2002-393059P	P	20020628		
	US 2002-407255P	P	20020830		
	US 2003-440252P	P	20030114		
	US 2003-428192	A2	20030502		
	WO 2003-US13765	W	20030502		
	US 2003-703816	A2	20031107		
OS	MARPAT 140:213577				
GI					



AB The present invention relates to phosphate-binding compds. that find use in binding, detecting and isolating phosphorylated target mols. including the subsequent identification of target mols. that interact with phosphorylated target mols. or mols. capable of being phosphorylated. A binding solution is provide that comprises a phosphate-binding compound, an acid and a metal ion wherein the metal ion simultaneously interacts with an exposed phosphate group on a target mol. and the metal chelating moiety of the phosphate-binding compound forming a bridge between the phosphate-binding compound and a phosphorylated target mol. resulting in a ternary complex. The binding solution of the present invention finds use in binding and detecting immobilized and solubilized phosphorylated target mols., isolation of phosphorylated target mols. from a complex mixture and aiding in proteomic anal. wherein kinase and phosphatase substrates and enzymes can be identified. A human MRC-5 lung fibroblast cell lysate protein mixture was separated by two-dimensional gel electrophoresis. The gel was fixed and then phosphoproteins were stained with a solution containing 50 mM NaOAc, pH 4.0, 250 mM NaCl, 20% volume/volume 1,2-propanediol, 1  $\mu$ M rhodamine-BAPTA chelating compound I, and 1  $\mu$ M gallium chloride.

RE.CNT 208 THERE ARE 208 CITED REFERENCES AVAILABLE FOR THIS RECORD.  
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L10 ANSWER 5 OF 9 CAPLUS COPYRIGHT 2007 ACS on STN

AN 2003:619840 CAPLUS

DN 139:257650

TI Quantitative analysis of protein phosphorylation status and protein kinase activity on microarrays using a novel fluorescent phosphorylation sensor dye

AU Martin, Karen; Steinberg, Thomas H.; Cooley, Laurie A.; Gee, Kyle R.; Beechem, Joseph M.; Patton, Wayne F.

CS Molecular Probes, Eugene, OR, USA

SO Proteomics (2003), 3(7), 1244-1255

CODEN: PROTC7; ISSN: 1615-9853

PB Wiley-VCH Verlag GmbH & Co. KGaA

DT Journal

LA English

AB Ultrasensitive detection of minute amts. of phosphorylated proteins and peptides is a key requirement for unraveling many of the most important signal transduction pathways in mammalian systems. Protein microarrays are potentially useful tools for sensitive screening of global protein expression and post-translational modifications, such as phosphorylation. However, the anal. of signaling pathways has been hampered by a lack of reagents capable of conveniently detecting the targets of protein kinases. Historically, phosphorylation detection methods have relied upon either radioisotopes (( $\gamma$ -<sup>32</sup>P)ATP( $\gamma$ -<sup>33</sup>P)ATP labeling) or phosphoamino acid-selective antibodies. Both of these methods suffer from relatively well-known shortcomings. In this study, a small mol. fluorophore phosphosensor technol. is described, referred to as Pro-Q Diamond dye, which is capable of ultrasensitive global detection and quantitation of phosphorylated amino acid residues in peptides and proteins displayed on microarrays. The utility of the fluorescent Pro-Q Diamond phosphosensor dye technol. is demonstrated using phosphoproteins and phosphopeptides as well as with protein kinase reactions performed in miniaturized microarray assay format. Instead of applying a phosphoamino acid-selective antibody labeled with a fluorescent or enzymic tag for detection, a small, fluorescent probe is employed as a universal sensor of phosphorylation status. The detection limit for phosphoproteins on a variety of different com. available protein array substrates was found to be 312-625 fg, depending upon the number of phosphate residues. Characterization of the enzymic phosphorylation of immobilized peptide targets with Pro-Q Diamond dye readily permits differentiation between specific and non-specific peptide labeling at picogram to subpicogram levels of detection sensitivity.

RE.CNT 21 THERE ARE 21 CITED REFERENCES AVAILABLE FOR THIS RECORD  
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L10 ANSWER 6 OF 9 CAPLUS COPYRIGHT 2007 ACS on STN

AN 2003:619829 CAPLUS

DN 139:273181

TI Global quantitative phosphoprotein analysis using multiplexed proteomics technology

AU Steinberg, Thomas H.; Agnew, Brian J.; Gee, Kyle R.;  
Leung, Wai-Yee; Goodman, Terrie; Schulenberg, Birte; Hendrickson, Jill;  
Beechem, Joseph M.; Haugland, Richard P.; Patton, Wayne F.

CS Molecular Probes, Eugene, OR, USA

SO Proteomics (2003), 3(7), 1128-1144

CODEN: PROTC7; ISSN: 1615-9853

PB Wiley-VCH Verlag GmbH & Co. KGaA

DT Journal

LA English

AB Systematic parallel anal. of the phosphorylation status of networks of interacting proteins involved in the regulatory circuitry of cells and tissues is certain to drive research in the post-genomics era for many years to come. Reversible protein phosphorylation plays a critical regulatory role in a multitude of cellular processes, including alterations in signal transduction pathways related to oncogene and tumor suppressor gene products in cancer. While fluorescence detection methods are likely to offer the best solution to global protein quantitation in proteomics, to date, there has been no satisfactory method for the specific and reversible fluorescent detection of gel-separated phosphoproteins from complex samples. The newly developed Pro-Q Diamond phosphoprotein dye technol. is suitable for the fluorescent detection of phosphoserine-, phosphothreonine-, and phosphotyrosine-containing proteins directly in SDS (SDS)-polyacrylamide gels and two-dimensional (2-D) gels. Addnl., the technol. is appropriate for the determination of protein kinase and phosphatase substrate preference. Other macromols., such as DNA, RNA, and sulfated glycans, fail to be detected with Pro-Q Diamond dye. The staining procedure is rapid, simple to perform, readily reversible and fully compatible with modern microchem. anal. procedures, such as matrix-assisted laser desorption/ionizationtime of flight (MALDI-TOF) mass spectrometry. Pro-Q Diamond dye technol. can detect as little as 1-2 ng omagnetic nanoparticles conjugated with  $\beta$ -casein, a pentaphosphorylated protein, and 8 ng of pepsin, a monophosphorylated protein. Fluorescence signal intensity correlates with the number of phosphorylated residues on the protein. Through combination of Pro-Q Diamond phosphoprotein stain with SYPRO Ruby protein gel stain, Multiplexed Proteomics technol. permits quant., dichromatic fluorescence detection of proteins in 2-D gels. This evolving discovery platform allows the parallel determination of protein expression level changes and altered

post-translational modification patterns within a single 2-D gel experiment

The linear responses of the fluorescence dyes utilized, allow rigorous quantitation of changes over an unprecedented 500-1000-fold concentration

range.

RE.CNT 56 THERE ARE 56 CITED REFERENCES AVAILABLE FOR THIS RECORD  
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L10 ANSWER 7 OF 9 CAPLUS COPYRIGHT 2007 ACS on STN

AN 2003:395448 CAPLUS

DN 140:124749

TI Strategies and solid-phase formats for the analysis of protein and peptide phosphorylation employing a novel fluorescent phosphorylation sensor dye

AU Martin, Karen; Steinberg, Thomas H.; Goodman, Terrie; Schulenberg, Birte; Kilgore, Jason A.; Gee, Kyle R.; Beechem, Joseph M.; Patton, Wayne F.

CS Molecular Probes, Inc., Eugene, OR, 97402, USA

SO Combinatorial Chemistry and High Throughput Screening (2003), 6(4), 331-339

CODEN: CCHSFU; ISSN: 1386-2073

PB Bentham Science Publishers Ltd.

DT Journal

LA English

AB Protein kinases represent one of the largest families of regulatory enzymes, with more than 2,000 of them being encoded for by the human genome. Many cellular processes are regulated by the reversible phosphorylation of proteins and upwards of 30% of the proteins comprising the eukaryotic proteome are likely to be phosphorylated at some point during their existence. In the past, anal. of global protein phosphorylation has been accomplished through radiolabelling of samples with inorg. <sup>32</sup>P or [ $\gamma$ -<sup>32</sup>P] ATP. The approach is limited to specimens amenable to radiolabelling and poses certain safety and disposal problems. Alternatively, immunodetection with antibodies to the common phosphoamino acids may be employed, but the antibodies are relatively expensive and exhibit limited specificity and a certain degree of cross-reactivity. Pro-Q Diamond dye is a new fluorescent phosphosensor technol. suitable for the detection of phosphoserine-, phosphothreonine- and phosphotyrosine-containing proteins directly in isoelec. focusing gels, SDS-polyacrylamide gels and two-dimensional gels. Addnl., the technol. is appropriate for the detection of phosphoproteins or phosphopeptides arrayed on protein chips or affixed to beads. Dye-stained proteins and peptides can be excited with a laser-based light source of 532 or 543 nm or with a xenon-arc lamp-based system equipped with appropriate band pass filters. Alternatively, UV light of about 302 nm may be employed, providing that sufficiently long exposure times are used to collect the fluorescence signal. Pro-Q Diamond dye emits maximally at approx. 580 nm. The fluorescence-based detection technol. is easy to conduct, cost effective and allows rapid large-scale screening of protein and peptide phosphorylation in a variety of solid-phase assay formats.

RE.CNT 26 THERE ARE 26 CITED REFERENCES AVAILABLE FOR THIS RECORD  
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L10 ANSWER 8 OF 9 CAPLUS COPYRIGHT 2007 ACS on STN

AN 1997:213477 CAPLUS

DN 126:273770

TI *Xenopus* actin depolymerizing factor/cofilin (XAC) is responsible for the turnover of actin filaments in *Listeria monocytogenes* tails

AU Rosenblatt, Jody; Agnew, Brian J.; Abe, Hiroshi; Bamburg, James R.; Mitchison, Timothy J.

CS Department of Biochemistry, University of California, San Francisco, CA, 94143, USA

SO Journal of Cell Biology (1997), 136(6), 1323-1332

CODEN: JCLBA3; ISSN: 0021-9525

PB Rockefeller University Press

DT Journal

LA English

AB In contrast to the slow rate of depolymn. of pure actin in vitro, populations of actin filaments in vivo turn over rapidly. Therefore, the rate of actin depolymn. must be accelerated by one or more factors in the cell. Since the actin dynamics in *Listeria monocytogenes* tails bear many similarities to those in the lamellipodia of moving cells, we have used *Listeria* as a model system to isolate factors required for regulating the rapid actin filament turnover involved in cell migration. Using a cell-free *Xenopus* egg extract system to reproduce the *Listeria* movement seen in a cell, we depleted candidate depolymg. proteins and analyzed the effect that their removal had on the morphol. of *Listeria* tails. Immunodepletion of *Xenopus* actin depolymg. factor (ADF)/cofilin (XAC) from *Xenopus* egg exts. resulted in *Listeria* tails that were approx. five times longer than the tails from undepleted exts. Depletion of XAC did not affect the tail assembly rate, suggesting that the increased tail length was caused by an inhibition of actin filament depolymn. Immunodepletion of *Xenopus* gelsolin had no effect on either tail length or assembly rate. Addition of recombinant wild-type XAC or chick ADF protein to XAC-depleted exts. restored the tail length to that of control exts., while addition of mutant ADF S3E that mimics the phosphorylated, inactive form of ADF did not reduce the tail length. Addition of excess wild-type XAC to *Xenopus* egg exts. reduced the length of *Listeria* tails to a limited extent. These observations show that XAC but not gelsolin is essential for depolymg. actin filaments that rapidly turn over in *Xenopus* exts. We also show that while the depolymg. activities of XAC and *Xenopus* extract are effective at depolymg. normal filaments containing ADP, they are unable to completely depolymerize actin filaments containing AMPPNP, a slowly hydrolyzible ATP analog. This observation suggests that the substrate for XAC is the ADP-bound subunit of actin and that the lifetime of a filament is controlled by its nucleotide content.

L10 ANSWER 9 OF 9 CAPLUS COPYRIGHT 2007 ACS on STN  
AN 1995:707889 CAPLUS  
DN 123:105683  
TI Reactivation of phosphorylated actin depolymerizing factor and  
identification of the regulatory site  
AU Agnew, Brian J.; Minamide, Laurie S.; Bamberg, James R.  
CS Dep. Biochem. Mol. Biol., Colorado State Univ., Fort Collins, CO, 80523,  
USA  
SO Journal of Biological Chemistry (1995), 270(29), 17582-7  
CODEN: JBCHA3; ISSN: 0021-9258  
PB American Society for Biochemistry and Molecular Biology  
DT Journal  
LA English  
AB Actin depolymerizing factor (ADF) occurs naturally in two forms, one of which  
contains a phosphorylated Ser and does not bind G-actin or  
depolymerize F-actin. Removal of this phosphate in vitro by alkaline  
phosphatase restores full F-actin depolymerizing activity. To identify the  
phosphorylation site, [32P]pADF was purified and digested with  
endoproteinase Lys-C. The digest contained only one 32P-labeled peptide.  
Further digestion with endoproteinase Asp-N and mass spectrometric anal.  
showed that this peptide came from the N terminus of ADF. Alkaline  
phosphatase treatment of one Asp-N peptide (mass 753) converted it to a  
peptide of mass 673, demonstrating that this peptide contains the  
phosphate group. Tandem mass spectrometric sequence anal. of this peptide  
identified the phosphorylated Ser as the encoded Ser3 (Ser2 in  
the processed protein). HeLa cells, transfected with either chick  
wild-type ADF cDNA or a cDNA mutated to code for Ala in place of Ser24 or  
Thr25, express and phosphorylate the exogenous ADF. Cells also expressed  
high levels of mutant ADF when Ser3 was deleted or converted to either Ala  
or Glu. However, none of these mutants was phosphorylated,  
confirming that Ser3 in the encoded ADF is the single in vivo regulatory  
site.



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L2      1412 SEA ABB=ON  PLU=ON  L1(L) (ISOLAT# OR SEPARAT# OR CHROMATOGRAPH
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L3      89 SEA ABB=ON  PLU=ON  L2 AND (GALLIUM OR GA OR IRON OR FE OR
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L4      7 SEA ABB=ON  PLU=ON  L3 AND (METAL(L) CHELAT# OR BAPTA OR IDA OR
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        D 1-7 BIB ABS
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        E GEE KYLE/AU
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        E MARTIN VLADIMIR/AU
L7      25 SEA ABB=ON  PLU=ON  ("MARTIN VLADIMIR"/AU OR "MARTIN VLADIMIR
        V"/AU)
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        D QUE L8 STAT
        D QUE L9 STAT
        D BIB ABS
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